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No Impact of Total or Myeloid Cd34+ Cell Numbers on Neutrophil Engraftment and Transplantation-Related Mortality after Allogeneic Pediatric Bone Marrow Transplantation

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A B S T R A C T

Although the influence of transplanted bone marrow (BM) CD34+ cells on neutrophil engraftment (NE) and transplantation outcomes has been discussed controversially, thresholds between 2 and 4 × 10^6/kg CD34+ cells are commonly accepted. This has substantial consequences for a donor in terms of BM volume to be collected, which frequently covers up to 15 to 20 mL/kg. As the BM CD34+ compartment contains varying fractions of CD34+/CD19+ B lymphoid progenitors, we tested the hypothesis that the infused CD34+/CD45dim/CD19-/CD10- myeloid stem cells might reliably predict NE in 94 children who received BM from 37 HLA-identical sibling donors (MSD) and 57 matched unrelated donors after myeloablative conditioning. The grafts contained a median of 3.6 × 10^6/kg total CD34+ cells, which consisted of a median of 73% myeloid CD34+ cells and 27% B lymphoid progenitors. Grafts from donors <15 years old yielded significantly lower myeloid fractions compared with grafts from older donors ($P < .001$). All patients achieved sustained NE after median 20 (range, 11 to 40) days. By multivariate analysis, neither the number of total CD34+ cells ($P = .605$) nor of myeloid CD34+ cells ($P = .981$) correlated with NE, whereas transplantation from MSD (hazard ratio [HR] 3.51; $P = .019$) and the administration of granulocyte colony–stimulating factor (HR 2.24; $P = .002$) remained independent factors associated with earlier NE. Furthermore, neither total nor myeloid CD34+ cell quantities were associated with incidences of severe infections before NE ($P = .271$ and $P = .132$) or transplantation-related mortality (TRM) at day +100 ($P = .294$ and $P = .490$). Taking into account that the number of transplanted total CD34+ or myeloid CD34+ cells does not seem to have a relevant impact on time to NE, sepsis rates, or TRM, the need of certain threshold cell numbers should be revisited, at least for pediatric MSD.

INTRODUCTION

The assumption that the number of transplanted CD34+ cells has an impact on engraftment kinetics seems obvious, and several studies described a correlation between bone marrow (BM) CD34+ cell content and the kinetics of neutrophil engraftment (NE) [1-6]. Others, however, did not find such a correlation [7-9]. Discrepant findings concerning the relation between CD34+ cell dose and engraftment kinetics have also been published in the context of peripheral stem cell (PBSC) transplantation [2,10-16]. The inconsistency of these findings might be explained by heterogeneous patient and transplantation characteristics and the varying content of myeloid and lymphoid progenitors within the CD34+ fraction [17-19].

Akashi et al. reported that common myeloid progenitors give rise to all myeloid lineages [20-22], which would justify the assumption that the myeloid CD34+ subset might better predict NE than the number of all CD34+ cells transplanted.

Since 2000, our 4-color CD34+ enumeration routine has included quantification of CD34+/CD19+ B lymphoid progenitor cells and of myeloid subsets (CD34+/CD45dim/CD19-/CD10-) [18]. In this retrospective analysis, the number of infused myeloid stem cells was correlated with NE in 100 consecutive pediatric patients who had received donor BM after myeloablative conditioning. Next, graft contents, as well as NE, were correlated with sepsis rates during neutropenia and day +100 transplantation-related mortality (TRM) to evaluate any clinical relevance.

PATIENTS AND METHODS

Patients and Transplantation Characteristics

To eliminate confounding factors, such as reduced-intensity conditioning, ex vivo T cell depletion, and PBSC or cord blood grafts, 100 consecutive children and adolescents receiving unmanipulated BM after myeloablative conditioning between 2000 and 2012 were included in the analysis. Four patients who had received granulocyte transfusions before engraftment, 1 patient with overt relapse on day +28, and 1 patient who died on day +11 were excluded. The median recipient age was 11.7 years (range, 1.0 to 23.1 years). All patients were treated for hemato-oncological diseases, with acute lymphoblastic or myeloid leukemia accounting for the vast majority. Fifty-nine patients (63%) received a total body irradiation–based conditioning regimen and 35 (37%) received a busulfan-based chemotherapy,

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according to disease-specific standard European protocols. Donors were HLA-identical/matched sibling donors (MSD) in 37 patients (39%), and 57 (61%) underwent transplantation from matched unrelated donors (MUD). HLA typing was performed at the allele level for HLA-A, -B, -C, -DRB, and -DRQ for all donor-recipient pairs, revealing an HLA disparity in 30 unrelated donors. The median donor age was 32.7 years for MUD and 12.7 years for MSD ($P < .001$). Patient and transplantation characteristics are summarized in Table 1.

Graft-versus-host disease prophylaxis for patients who underwent transplantation from unrelated donors comprised ATG (Fresenius Biotech, Graefelfing, Germany; 20 mg/kg) or Thymoglobuline (Genzyme Polyclonals S.A.S., Marcy L'Etoile, France; 2.5 mg/kg) given on 3 consecutive days (days -3 to -1), serum-level-adjusted cyclosporine A (CyA) commencing on day -1, and short course methotrexate (MTX) on days +1, +3 and +6 (56 CyA+MTX; 1 CyA only). Patients who underwent transplantation from MSD primarily received chemoprophylaxis with CyA. In 5 patients, CyA had to be replaced by tacrolimus because of adverse effects.

Supportive measures included isolation of patients in laminar airflow units and transfusions of leukocyte-depleted and irradiated red blood cells or platelets from cytomegalovirus (CMV) -seronegative donors, according to our local policy. Based on pretransplantation serologic testing for herpes simplex or varicella zoster virus, seropositive patients received acyclovir (n = 52) as prophylactic therapy. CMV-positive recipients who underwent transplantation from CMV-negative donors received prophylactic gancyclovir (n = 27), and patients with CMV reactivation and detectable CMV DNA in the peripheral blood between days −7 and +60 were treated with gancyclovir or cidofovir (n = 4). Recombinant hematopoietic growth factors were not routinely used; only patients with severe bacterial or fungal infections before NE were treated with granulocyte-colony stimulating factor (G-CSF) at 5 μg/kg/day (n = 45).

Table 1
Patient and Transplant Characteristics

Characteristics	Value
Diagnosis	
ALL	51 (54%)
AML	17 (18%)
CML	9 (10%)
MDS	4 (4%)
JMML	3 (3%)
NHL	6 (6%)
Secondary AML	4 (4%)
Conditioning regimen	
TBI-based	59 (63%)
Chemotherapy-based	35 (37%)
Donor	
Matched sibling (MSD)	37 (39%)
Matched unrelated (MUD)	57 (61%)
1-AG-mm	26 (46%)
>1-AG-mm	4 (7%)
GVHD prophylaxis*	
CyA	38 (40%)
CyA + MTX	56 (60%)
Virus prophylaxis	
Acyclovir	52 (55%)
Gancyclovir	27 (29%)
Gancyclovir + cidofovir	4 (4%)
n.a.	11 (12%)
G-CSF before engraftment	
Yes	45 (48%)
No	46 (49%)
n.a.	3 (3%)
Age, median (range), yr	
Donor age (MSD)	12.7 (2.5-38.6)
Donor age (MUD)	32.7 (20.0-51.0)
Recipient age	11.7 (1.0-23.1)
Cellular graft content, median (range)	
Total CD34+ cells	3.6 (.4-17) × 10^6/kg
Myeloid CD34+ cells	2.4 (.4-10) × 10^6/kg
B lymphoid CD34+ cells	.9 (.1-7) × 10^6/kg
CD3 + cells	40 (4.5-133) × 10^6/kg

ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; JMML, juvenile myelo-monocytic leukemia; NHL, non-Hodgkin lymphoma; TBI, total body irradiation; MSD, matched sibling donor; MUD, matched unrelated donor; AG-mm, antigene mismatch; GVHD, graft-versus-host disease; CyA, cyclosporin A; FK506, tacrolimus; MTX, methotrexate; G-CSF, granulocyte colony−stimulating factor; n.a., not available.
Data presented are n (%) unless otherwise indicated.
* CyA replaced by Tacrolimus in 5 patients.

Graft Characteristics and Stem Cell Quantification

The BM from sibling donors was collected at our hospital by aspiration from posterior iliac crests under general anesthesia. Grafts from unrelated donors were harvested at the respective collection centers. In case of major or minor blood group incompatibility, grafts underwent a combined erythrocyte and plasma depletion by using an apheresis system.

All grafts were analyzed by flow cytometry at our local stem cell laboratory before transplantation. In case of erythrocyte or plasma depletion because of AB0 incompatibility, the stem cell number was determined after cell processing. In brief, the content of all CD34+ cells and of myeloid CD34+ cells (CD34+/CD45dim/CD19-) were determined by 4-color dual platform flow cytometry as described previously [23]. Normoblasts were defined as Syto 16 positive, CD45 negative, and CD71 positive. The WBC value obtained from the hematology analyzer was adjusted accordingly before calculation of CD34+ numbers. A FACSCalibur (BD Biosciences, San Jose, CA) was used for acquisition, and the Paint A Gate software (BD) for data evaluation. CD34+ cells were defined according to the International Society of Hematotherapy and Graft Engineering guidelines [24]. They were defined by their dim CD45 expression and by their position in the lympho-monocytic window in the forward-scattered light/side-scattered light dot plot. B lymphoid progenitor cells were determined by coexpression of CD34, as well as CD19 and by their smaller forward-scattered light properties (left lymphoid window).

Definition of Engraftment and Other Endpoints

NE was defined as the first of 3 subsequent days with more than .5 × 10^9/L absolute neutrophil cells in the peripheral blood. Chimerism analyses were performed on days +14, +21, +28, and +60, on 6 to 8 FACS-sorted WBC subpopulations (CD4+ and CD8 + T cells, CD19+ B cells, NK cells, monocytes, granulocytes, CD34+ cells, and normoblasts) using either fluorescence in situ hybridization for patients who underwent transplantation from sex-mismatched donors, or short tandem repeat PCR, in case of transplantation from donors of identical gender. According to a determined median time to NE of 20 days for the whole cohort, the rate of NE at day +20 was chosen as primary endpoint.

For sepsis analysis, only severe infections within the neutropenic period after transplantation were taken into account. Severe bacterial or fungal infections were considered when patients had symptoms of sepsis, overt septic shock according to published definitions [25], positive microbiological blood testing, or radiologic findings suggestive of bacterial or fungal pneumonia.

Death in continuous complete remission due to transplantation-associated complications within the first 100 days was considered an event for 100-day TRM.

Overall survival (OS) was defined as the time interval between transplantation and death from any cause. Patients surviving without event were censored at last follow-up, with a median follow-up time of 3.2 years (range, .1 to 11.2 years).

Statistical Analysis

To analyze the impact of stem cells dose on engraftment kinetics and other outcomes (OS, TRM, and sepsis), 3 stem cell groups were defined according to the content of total CD34+ cells and of myeloid CD34+ cells: group I with $< 3 \times 10^6$/kg total CD34+ or $< 2 \times 10^6$/kg myeloid CD34+cells; group II with ≥ 3 and $< 6 \times 10^6$/kg total CD34+ or ≥ 2 and $< 4 \times 10^6$/kg myeloid CD34+ cells; and group III with $\geq 6 \times 10^6$/kg total CD34+ cells or $\geq 4 \times 10^6$/kg myeloid CD34+ cells. Additional parameters investigated for their influence on engraftment and other outcomes included donor type, donor and recipient age, HLA disparity, conditioning regimen, use of G-CSF, and prophylactic treatment with antiviral drugs.

The univariate statistical analysis was done in several prospectively identified subgroups defined by donor type, HLA match, donor and recipient age, conditioning, and virus prophylaxis. The cumulative incidences of NE, TRM, and sepsis, and the probability of OS were analyzed according to the Kaplan-Meier method [26] and compared by the log-rank test. No competing risks had to be taken into account as all patients achieved NE by day +40 and only treatment-related deaths occurred until day +100. The cumulative incidence of platelet engraftment (PE) was calculated according to the method by Kalbfleisch and Prentice and compared using Gray's test [27]. For other than time-to-event variables, the chi-square test was used to compare groups for categorical variables and the Wilcoxon rank-sum test (Kruskal-Wallis test for more than 2 populations) was used for continuous variables. The impact of the predefined confounding factors on the defined outcomes was investigated using a multivariate Cox regression with time-

dependent covariates [28,29]. The analysis of the variable "G-CSF use before engraftment" considered its time-dependent character by using a Cox regression in both univariate and multivariate analysis. The statistical analysis was done with SAS System V9.2 (2008, SAS Institute, Cary, NC). All *P* values <.05 were considered significant.

RESULTS

Cellular Graft Composition

The grafts contained a median of 3.6 × 10^6/kg total CD34+ cells (range, .4 to 17 × 10^6/kg; data available in all 94 patients), 2.4 × 10^6/kg myeloid CD34+ cells (range, .4 to 10 × 10^6/kg; data missing in 5 patients), and .9 × 10^6/kg B lymphoid CD34+ cells (range, .1 to 7 × 10^6/kg; data missing in 8 patients). Twenty-nine patients received <2 × 10^6/kg myeloid CD34+ cells. The CD34+ cell population of all grafts consisted of a median of 73% myeloid CD34+ cells (range, 33% to 100%) and 27% CD34/CD19 coexpressing B lymphoid progenitors (range, 0 to 67%). The median graft content of CD3 + T cells was 40 × 10^6/kg (range, 4.5 to 133 × 10^6/kg; data not available in 3 patients). Of note, the median number of myeloid CD34+ cells and of B lymphoid progenitors and their distribution differed significantly within different donor age groups. Grafts from donors < 15 years old yielded significantly lower myeloid fractions compared with grafts from older donor groups ($P < .001$) (Figure 1). As expected, donor gender did not correlate with graft composition ($P = .420$).

Neutrophil Engraftment

All 94 patients achieved sustained NE after a median of 20 days (range, 11 to 40 days). The median time to NE of patients with early (before day +20) and late (after day +20) engraftment was 18 and 24 days, respectively. Engraftment was confirmed by chimerism analysis, which revealed >98% donor chimerism in the myeloid cell subsets in all patients. We did not observe any correlation between the total numbers of all CD34+ cells or of myeloid CD34+ cells infused and time to NE.

The median time to NE for the total CD34+ cell numbers was 22 (group I), 19 (group II), and 20 days (group III) ($P = .901$). Likewise, the median time to NE for the myeloid CD34+ numbers was 20 (group I), 19 (group II), and 21 days (group III) ($P = .492$). The total CD34+ cell content did not correlate with NE at day +20: the probabilities of NE at day +20 were 44% ± 8% (group I), 56% ± 8% (group II), and 59% ± 12% (group III) ($P = .788$). Similarly, the number of myeloid CD34+ cells did not correlate with the probabilities of NE at day +20: 52% ± 9% (group I), 54% ± 8% (group II), and 48% ± 11% (group III) ($P = .665$) (Figure 2). Of note, the median time to NE of the 29 patients who received < 2 × 10^6/kg myeloid CD34+ cells was 20 days, and it was18 days for 9 patients receiving <1 × 10^6/kg myeloid CD34+ cells.

By univariate analysis, the probability of NE at day +20 was significantly higher in patients who underwent transplantation from MSD compared with those who underwent transplantation from MUD (73% ± 7% versus 39% ± 6%, $P < .001$). The median time to NE was 18 days (range, 11 to 30) in patients who underwent transplantation from MSD and 23 days (range, 14 to 33) in patients who underwent transplantation from MUD ($P < .001$) (Table 2).

Earlier NE was observed in patients receiving G-CSF ($P = .010$) and in patients who underwent transplantation from younger donors ($P = .056$). Treatment with gancyclovir had a negative impact on NE ($P = .035$) compared with acyclovir, whereas the conditioning regimen (TBI-based versus busulfan-based regimen) ($P = .362$), recipient age ($P = .725$),

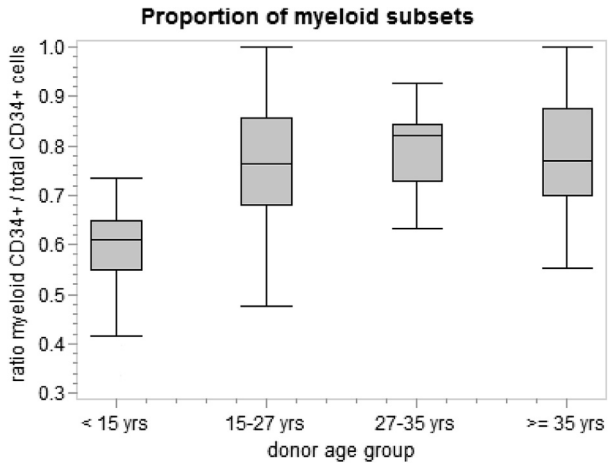

Figure 1. Proportion of myeloid CD34+ subsets out of total CD34+ cells within the donor age groups. The donor age groups' median content of myeloid CD34+ subsets and their percentage of total CD34+ cells were 2.55 × 10^6/kg (61%) for donors <15 years (n = 23), 1.80 × 10^6/kg (77%) for donors ≥ 15 and < 27 years (n = 24), 3.90 × 10^6/kg (82%) for donors ≥ 27 and < 35 years (n = 22), and 2.50 × 10^6/kg (77%) for donors ≥ 35 years (n = 25) ($P < .001$).

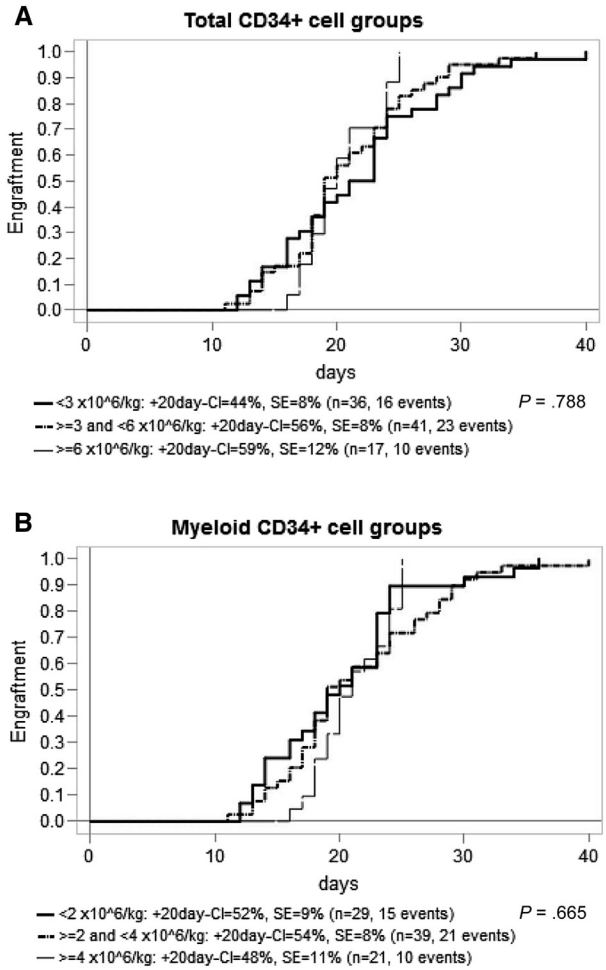

Figure 2. Probabilities of neutrophil engraftment at day +20 for patients of the respective total CD34+ cell groups (A) and myeloid CD34+ cell groups (B).

Table 2
Neutrophil Engraftment Probabilities and Median Engraftment Time

Variable	Number	Probability of ANC Engraftment at Day +20	*P* Value*	ANC Engraftment, Median (range), d	*P* Value†
Donor type			<.001		<.001
MSD	37	.73 ± .07		18 (11-30)	
MUD	57	.39 ± .06		23 (14-33)	
HLA typing results			.271		.087
HLA matched	64	.55 ± .06		19.5 (11-40)	
HLA mismatched	30	.47 ± .09		22.5 (16-36)	
Graft stem cell content					
Total CD34+ cells			.788		.901
$<3 \times 10^6$/kg	36	.44 ± .08		22 (12-40)	
≥ 3 and $< 6 \times 10^6$/kg	41	.56 ± .08		19 (11-36)	
$\geq 6 \times 10^6$/kg	17	.59 ± .12		20 (16-25)	
Myeloid CD34+ cells			.665		.492
$<2 \times 10^6$/kg	29	.52 ± .09		20 (12-36)	
≥ 2 and $< 4 \times 10^6$/kg	39	.54 ± .08		19 (11-40)	
$\geq 4 \times 10^6$/kg	21	.48 ± .11		21 (16-25)	
n.a.	5	-		-	
Conditioning			.362		.415
TBI-based	59	.47 ± .07		21 (11-36)	
Chemotherapy-based	35	.60 ± .08		19 (13-40)	
Donor age, yr			.056		.063
<15	23	.70 ± .10		18 (11-30)	
≥15 and <27	24	.50 ± .10		20.5 (12-36)	
≥27 and <35	22	.41 ± .10		21 (14-33)	
≥35	25	.48 ± .10		22 (13-40)	
Recipient age, yr			.725		.196
<7	22	.55 ± .11		20 (12-36)	
≥7 and <12	29	.52 ± .09		20 (13-30)	
≥12 and <16	23	.43 ± .10		23 (11-40)	
≥16	20	.60 ± .11		19 (12-24)	
Virus prophylaxis			.035		.022
Acyclovir	52	.60 ± .07		19 (11-36)	
Gancyclovir	31	.39 ± .09		23 (15-40)	
n.a.	11	-		-	

ANC indicates absolute neutrophil cells; MSD, matched sibling donor; MUD, matched unrelated donor; TBI, total body irradiation; n.a., not available.
* Comparison made with log-rank test.
† Comparison made with Wilcoxon rank-sum test.

and HLA disparity ($P = .271$) did not seem to have an impact on the probability of NE at day +20.

When patients who underwent transplantation from MSD and MUD were analyzed separately, the number of infused myeloid CD34+ cells did not correlate significantly with the probability of NE at day +20 ($P = .175$ and $P = .110$) (Figure 3). Furthermore, the number of infused total CD34+ cells did not correlate with the probability of NE at day +20 for patients who underwent transplantation from MSD ($P = .311$) but correlated with the probability of NE at day +20 for patients who underwent transplantation from MUD ($P = .023$). Of note, children who underwent transplantation from MUD were younger (median recipient age, 9.9 years versus 14.0 years; $P = .005$), had older donors than those who underwent transplantation from MSD (median donor age, 32.7 versus 12.7 years; $P < .001$), and hence, received higher doses of total and of myeloid CD34+ cells (50% of children who underwent transplantation from MSD received $<2 \times 10^6$/kg myeloid CD34+ cells versus 21% of patients who underwent transplantation from MUD; $P = .002$).

By multivariate analysis, transplantation from MSD (hazard ratio [HR], 3.51; 95% confidence interval [CI], 1.23 to 9.96; $P = .019$) and the administration of G-CSF (HR, 2.24; CI 1.35 to 3.73; $P = .002$) remained independent factors associated with earlier NE. In contrast, neither the numbers of total or of myeloid CD34+ cells, nor donor or recipient age, virus prophylaxis, or conditioning correlated significantly with the time to NE (Table 3). Because of the limited number of patients, the multivariate analysis was performed for the complete cohort but not for subgroups, and the results of the multivariate statistical analysis have to be regarded with caution.

Platelet Engraftment

Out of 91 patients with available PE information, 83 achieved stable PE (defined as thrombocyte recovery to $>20 \times 10^9$/L peripheral blood for at least 7 days without platelet transfusions) after a median of 31 (range, 13 to 100) days. Eight patients died before PE. The median time to PE for the total CD34+ cell numbers was 33.5 (group I), 34 (group II), and 26 days (group III) ($P = .103$); the median time to PE for the myeloid CD34+ numbers was 32 (group I), 36 (group II), and 29 days (group III) ($P = .451$). The total CD34+ cell content did not significantly correlate with PE at day +31: the probabilities of PE at day +31 were 45% ± 9% (group I), 41% ± 8% (group II), and 71% ± 11% (group III) ($P = .125$). Similarly, the number of myeloid CD34+ cells did not correlate with the probabilities of PE at day +31: 48% ± 10% (group I), 39% ± 8% (group II), and 62% ± 11% (group III) ($P = .498$) (Figure 4).

Sepsis during Neutropenia and Day +100 TRM

Forty-five patients (48%) experienced severe infections before engraftment. The number of transplanted total and myeloid CD34+ cells did not significantly correlate with the incidence of severe infections. Cumulative incidences of sepsis within the first 40 days after transplantation were 38% ± 9% (group I), 59% ± 8% (group II), and 38% ± 11% (group III) for patients receiving the respective myeloid

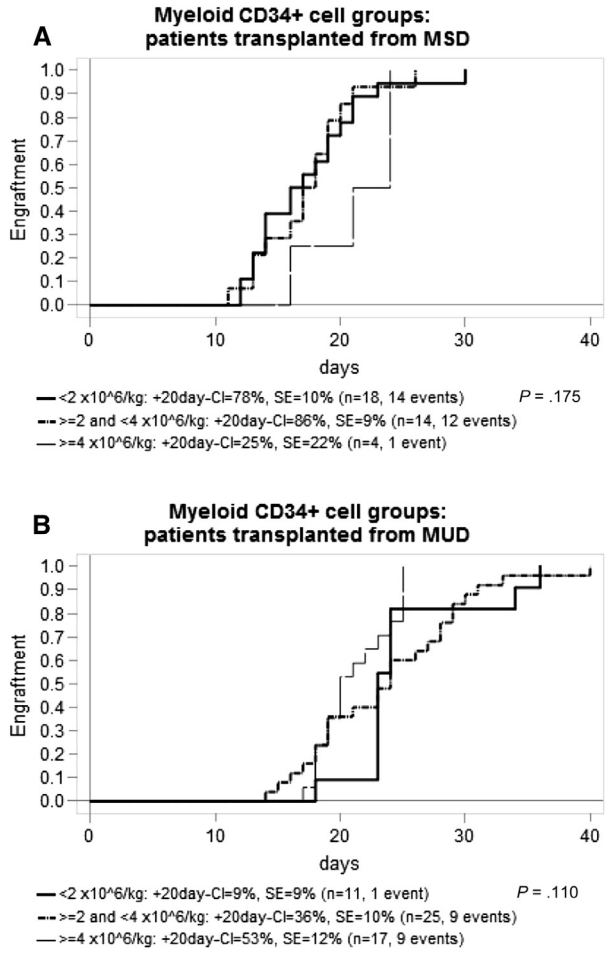

Figure 3. Probabilities of neutrophil engraftment at day +20 for patients of the respective myeloid CD34+ cell groups after transplantation from sibling donors (A) and unrelated donors (B).

CD34+ numbers ($P = .132$). There was a trend towards higher sepsis rates in patients who underwent transplantation from HLA-mismatched unrelated donors (60% ± 9%) and MUD (53% ± 7%) compared with patients who underwent transplantation from MSD (38% ± 8%) ($P = .178$). Other variables (donor or recipient age, conditioning regimen, virus prophylaxis, or G-CSF) did not significantly correlate with the occurrence of sepsis neither in univariate nor multivariate analysis (detailed results not shown). Only 1 patient (group III) experienced invasive aspergillosis.

The cumulative incidence of TRM at day +100 was 7% ± 3% for the complete cohort, and it did not correlate with total CD34+ cell numbers (group I: 3% ± 3%; group II: 12% ± 5%; and group III: 6% ± 6% [$P = .294$]) or with myeloid CD34+ cell numbers (group I: 3% ± 3%; group II: 10% ± 5%; and group III: 5% ± 5% [$P = .490$]) (Figure 5). None of the other parameters investigated (donor type, donor or recipient age, conditioning regimen, or virus prophylaxis) had a significant impact on day + 100 TRM. Because of the limited number of events, a multivariate analysis was not performed. Of note, NE within the first 20 days after transplantation did not correlate with day +100 TRM either: 5 of 49 patients with early NE and 2 of 45 patients with delayed NE died because of transplantation-associated reasons.

The estimates of 5-year OS were 81% ± 7%, 54% ± 9%, and 79% ± 11% ($P = .053$) for patients of the low, middle, and high total CD34+ group, along with 80% ± 8%, 58% ± 9%, and 68% ± 13% ($P = .195$) for the respective myeloid CD34+ groups. There was no difference in OS for children who underwent transplantation from either MSD or MUD (5-year OS: 69% ± 8% versus 69% ± 7%; $P = .863$). All other variables (donor type, donor or recipient age, conditioning, virus prophylaxis) did not significantly impact survival in univariate or multivariate analysis (detailed results not shown).

DISCUSSION

Based on numerous studies in autologous and allogeneic hematopoietic stem cell transplantation (HSCT), after

Table 3
Multivariable Regression Model for Neutrophil Engraftment

Analysis of Maximum Likelihood Estimates						
Parameter	Parameter Estimate	Standard Error	Chi- Square	*P* Value	Hazard Ratio	95% Hazard Ratio Confidence Limits
Total CD34+ cells						
$<3 \times 10^6$/kg	−.377	.728	.268	.605	.686	.165-2.856
≥ 3 and $<6 \times 10^6$/kg	.273	.552	.245	.621	1.314	.445-3.877
Myeloid CD34+ cells						
$<2 \times 10^6$/kg	.016	.691	.001	.981	1.017	.262-3.941
≥ 2 and $< 4 \times 10^6$/kg	.014	.490	.001	.977	1.014	.388-2.647
Donor						
MSD HLA identical	1.254	.532	5.550	.019	3.505	1.235-9.952
MUD HLA identical	.096	.346	.077	.782	1.101	.559-2.169
Donor age, yr						
<15	−.255	.607	.177	.674	.775	.236-2.544
<27	−.084	.431	.038	.845	.919	.395-2.140
<35	.183	.359	.259	.611	1.200	.594-2.428
Recipient age, yr						
<12	−.699	.488	2.055	.152	.497	.191-1.293
<16	−.830	.468	3.140	.076	.436	.174-1.092
<7	−.404	.573	.497	.481	.668	.217-2.053
Conditioning						
Chemotherapy based	.400	.333	1.436	.231	1.491	.776-2.866
Virus prophylaxis						
Acyclovir	.187	.289	.420	.517	1.206	.685-2.123
G-CSF						
G-CSF given	.808	.256	9.942	.002	2.243	1.358-3.706

G-CSF indicates granulocyte colony−stimulating factor; MSD, matched sibling donor; MUD, matched unrelated donor.

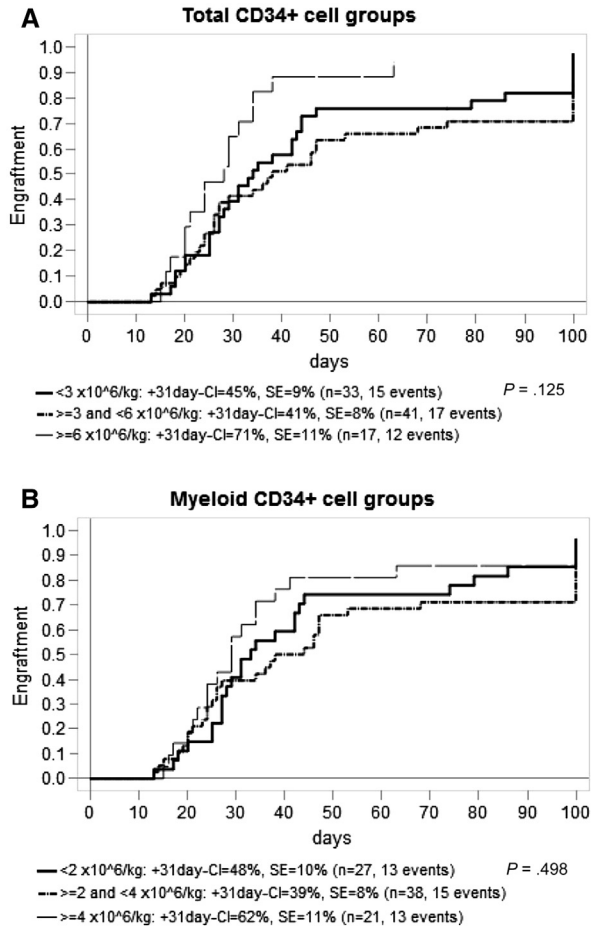

Figure 4. Probabilities of platelet engraftment at day +31 for patients of the respective total CD34+ cell groups (A) and myeloid CD34+ cell groups (B).

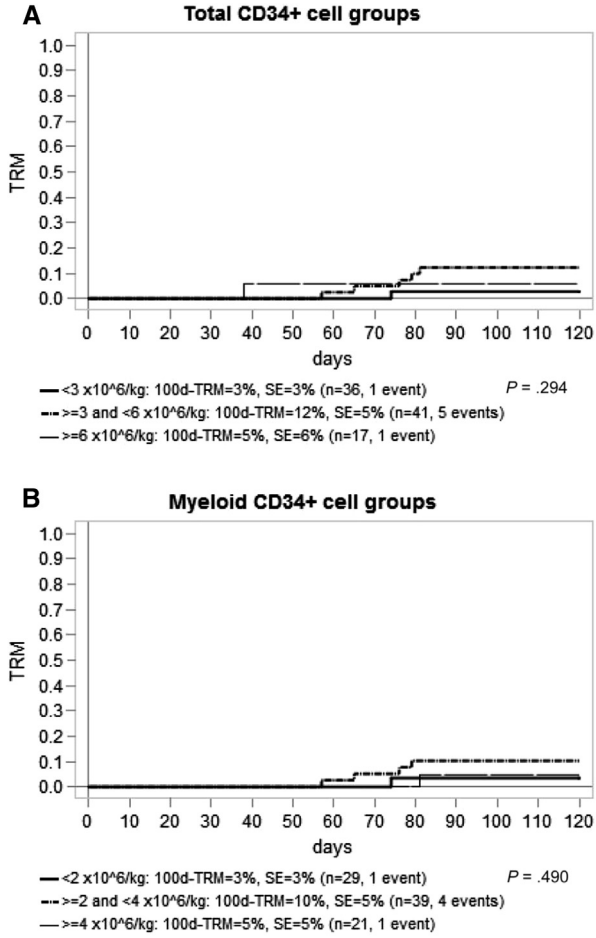

Figure 5. Cumulative incidences of transplantation-related mortality (TRM) at day +100 for patients of the respective total CD34+ cell groups (A) and myeloid CD34+ cell groups (B).

myeloablative or reduced-intensity conditioning, the graft stem cell content is considered a major determinant of various transplantation outcomes, including engraftment kinetics and TRM. There is no doubt that in the context of unrelated cord blood grafts, the CD34 cell dose plays a substantial role for both engraftment probability and kinetics, as well as for TRM [30]. It has been shown in various randomized studies that NE is significantly faster with PBSC grafts that contain an up to 5-fold higher number of CD34+ cells as compared with BM [31-34]. Several studies, however, which support the impact of the transplanted cell dose on NE, have included both BM and PBSC grafts [3,14], not taking into account that the cell source itself might have an influence on engraftment kinetics. In the context of PBSC grafts, the correlation between CD34+ cell dose and NE is inconsistent [10,13,15]. A functional correlation of the BM CD34+ cell content with NE has been described by some groups [1,4,5], whereas others reported no association for both related or unrelated HSCT [7-9]. Consequently, several minimal threshold CD34+ cell doses between 2 and 4 $\times$ 10^6/kg have been proposed [1,2,5].

The heterogeneity of the CD34+ cell compartment described for different graft sources [17] including BM grafts [18,19], led us to evaluate the correlation of transplanted myeloid CD34+ cell subsets with NE in a homogenous group of pediatric patients who underwent transplantation with unmanipulated BM from related or unrelated donors after myeloablative conditioning.

The myeloid CD34+ cell fraction was lowest in grafts from donors < 15 years old and increased with donor age. An age-dependent increase of myeloid CD34+ subsets, accompanied by a relative decrease in lymphoid progenitors was reported in BM of mice [35-37]. Moreover, an age-dependent decrease in B lymphoid progenitors in human BM has been proposed [38,39]. Recently, the persistence of myeloid progenitors at the same level and a declining frequency of lymphoid progenitors with increasing age were suggested after phenotypic analysis of 48 BM samples from donors older than 14 years [40].

In our study, the total CD34+ cell number did not correlate significantly with engraftment kinetics. This is in contrast to the findings of Bittencourt et al. in 212 patients who underwent transplantation with unmanipulated BM from MSD [1]. However, this study included both adult and pediatric patients, and donor age was not included as a variable in their multivariate analysis despite a wide range (1.2 to 64.8 years). Marley et al. found an age-dependent relative increase of myeloid progenitors in human BM, whereas the capacity to replicate declines with increasing age [41]. It was furthermore reported that the repopulating capacity of human BM CD34+ cells from older donors is remarkably reduced after injection into NOD/SCID/interleukin-2 receptor gamma-chain-null mice, and that a substantial decrease in the generation of myeloid cell lines was observed with rising donor age [40]. Hence, the reported correlation of total

CD34+ cell numbers with NE might be explained by the fact that younger donors usually have more CD34+ cells/mL BM, albeit with a high proportion of B cell precursors [42]. Our finding that the number of infused total CD34+ cell/kg did not correlate with NE confirms previous studies [7-9].

The present data refute our original hypothesis that the number of myeloid stem cells infused might better predict NE than the number of all CD34+ cells: there was no significant correlation of the number of infused myeloid CD34+ cells with NE in our pediatric patient cohort. These results are in line with the report by Collins, who did not find a correlation of myeloid or lymphoid subsets of BM grafts with NE in a cohort including foremost adults who underwent transplantation from unrelated donors after myeloablative and reduced-intensity conditioning [7]. Notably, NE in children who underwent transplantation from MUD was significantly delayed as compared with recipients of grafts from MSD, although they received higher stem cell doses. This finding might be explained by a combined effect of higher donor age, pretransplantation serotherapy, and posttransplantation methotrexate in patients who underwent transplantation from MUD.

We are aware that our analysis has some limitations that should be addressed. At first, differences in engraftment kinetics might not have gained significance because of a limited number of patients who received low numbers of total or myeloid CD34+ cells. For example, only 9 patients received $<1 \times 10^6$/kg myeloid CD34+ cells. Therefore, we cannot rule out that the stem cell doses for patients of group I were above a certain threshold required for the confirmation of an evident dose-response relationship. Furthermore, several studies on hematopoietic recovery after HSCT reported that platelet recovery is strongly influenced by transplanted stem cell numbers, in some reports even stronger than NE [1-4,6,17,43]. Therefore, the transplantation of lower stem cell numbers might postpone PE. Of note, we found no significant correlation of total and myeloid stem cell numbers with PE in our pediatric cohort (Figure 4). These findings are in line with previous reports [7-9]. However, we found a nonsignificant trend towards a faster platelet recovery after transplantation of very high ($\geq 6 \times 10^6$/kg) numbers of total CD34+ cells but not of myeloid CD34+ cells.

As shown by others, the administration of G-CSF significantly enhanced the rate of NE [44,45], although G-CSF was only administered in case of severe bacterial infections. Of note, the frequency of G-CSF administration was similar in patients who underwent transplantation from either siblings or MUD. The incidence of severe bacterial and fungal infections did not correlate with the number of infused total and myeloid CD34+ cells.

The general belief that earlier NE is associated with a decrease in TRM and, thus, improved OS is the rationale for the paradigm of "the more the better," and several authors have correlated the number of CD34+ cells/kg with TRM and OS [1,2,14]. In the present study, neither TRM at day +100 nor the 5-year OS were significantly influenced by total or myeloid CD34+ cell doses. Of note, in our study, NE at day +20 did not seem to have a significant impact on day +100 TRM. In a retrospective analysis in adults comparing PBCSC to BM, Bittencourt et al. did not find a difference in TRM either, despite a significantly earlier NE [46]. Late NE has been described as risk factor for invasive aspergillosis in adults undergoing HSCT from alternative donors [47]. Late NE, however, was defined in this study as NE after day +40, whereas in our patient cohort, the median time to NE of those with late (after day +20) engraftment was 24 days, and only 1 patient had NE as late as day +40.

The belief that a higher number of infused BM stem cells correlates with earlier NE and, hence, reduces TRM has significant consequences for BM donors and, in particular, for pediatric sibling donors. The common practice of harvesting at least 1 to 2×10^8 nucleated cells per kg recipient implicates prolonged general anesthesia, a higher number of BM biopsies, and an increased need of transfusion. Van Walraven et al. recently reported that BM volumes exceeding 15 mL/kg were harvested in 65% of sibling donors under the age of 13 years, and that larger volumes were harvested from donors with older recipients [48]. Of note, in their retrospective analysis, all patients receiving less than 1×10^8 nucleated cells per kg engrafted, and none of them died from transplantation-related causes. Taking into account the fact that the cell number infused does not seem to have a relevant impact on time to engraftment and on TRM in children, and the increasing ethical concerns about bone marrow donation of pediatric sibling donors, the need of certain threshold cell numbers should be revisited.

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